DETERMINATION OF TOTAL ALKALOIDS AND TOTAL NITROGEN BY NEAR INFRARED REFLECTANCE SPECTROSCOPY

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SUMMARY

Near infrared reflectance spectroscopy (NIR) can be used to increase the rapidity with which the level of chemical constituents in tobacco can be determined.

One hundred and twenty samples of Virginia tobaccos were scanned with computerized NIR spectrophotometer (TECHNICON 450 R +) to study the relationship of intensity of reflectance spectra to the total alkaloids and total nitrogen content of the samples.
A multiple linear regression model was used to select the most appropriate wavelengths for the measurements.

The equations for

TOTAL ALKALOIDS (%) = 1.862 + 1.77 (log 1/R)546 - 44.93 (log 1/R)2310 + 220.7 (log 1/R)2270 -222.0 (log 1/R)2230 + 691.6 (log 1/R)1818 - 512.2 (log 1/R)1759 + 8.614 (log 1/R)1940 - 609.2 (log 1/R)1734 - 34.2 (log 1/R)1722 + 807.8 (log 1/R)1680

and for

TOTAL NITROGEN (%) = 4.941 + 1.677 (log 1/R)546 - 40.64 (log 1/R)2310 - 61.03 (log 1/R)2208 + 118.15 (log 1/R)2180 + 317.96 (log 1/R)1982 - 346.95 (log 1/R)1818 +805.3 (log 1/R)1778 - 92.66 (log 1/R)1759 + 13.8 (log 1/R)1940 + 560.32 (log 1/R)1734 - 617.1 (log 1/R)1722 - 34.15 (log 1/R)1445

give results in good agreement with standard chemical methods.The coefficient of correlation for total alkaloids (R) was 0.9908 and the standard error of calibration was +/- 0.1681. For total nitrogen coefficient of correlation R was 0.995 and the standard error was +/- 0.022.

Using these equations the total alkaloids and the total nitrogen in an independent set of samples could be determined with relative error, against wet chemistry, of +/- 6.2 % for total alkaloids and +/- 5.5 % for total nitrogen.

INTRODUCTION

Requirements concerning a knowledge of the chemical and physical characteristics of raw materials and/or final products are very important in any industry, and thus also in the tobacco processing industry. There are numerous laboratory techniques which, by their range of application, their accuracy and their speed, meet the requirements of the industry.
Methods and/or techniques such as Liquid Chromatography, Gas Chromatography, AAS or emission methods such as ICP as well as automatized chemical methods are accurate, relatively fast and have a wide range of application. The step which slows down analyses carried out by the above mentioned techniques is the preparation of samples. During sample preparation, the sample has to be destroyed, dissolved and/or extracted, so this step of the analysis is a factor limiting the speed with which the analysis is performed.

An analysis technique which has been attracting attention since the early sixties is the analysis of the chemical composition of a sample based on absorption changes of reflected near infrared radiation from the sample. The advantages of this technique are the shorter time needed to prepare the samples, the speed of the analysis itself, its accuracy and reproducibility, as well as its non-destructiveness.

Analysis of the total nitrogen, alkaloids, protein nitrogen, total reducing sugars, polyphenols and some inorganic components have already been made on tobacco (1 - 4).

METHODS, MATERIALS AND INSTRUMENTS

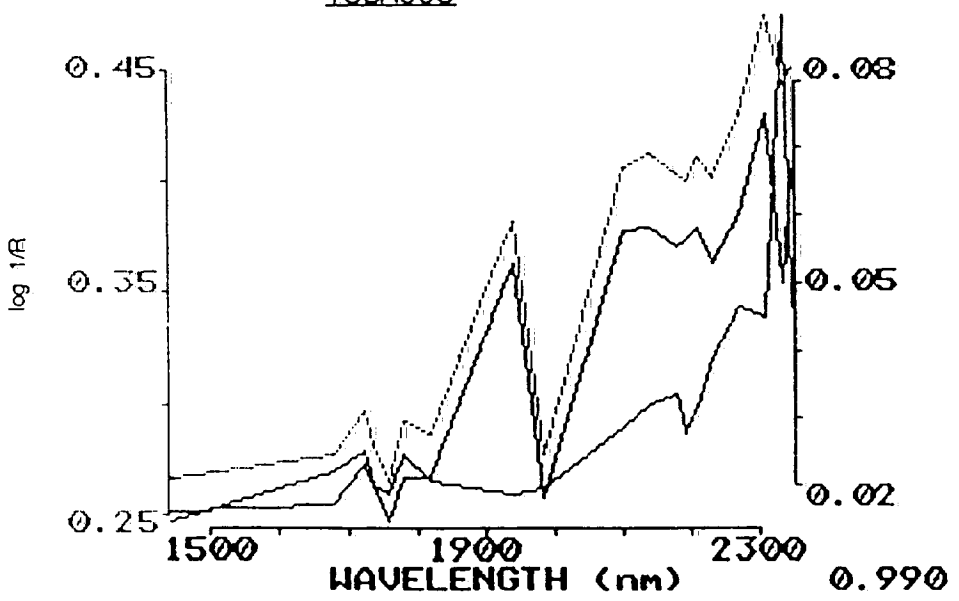
A TECHNICON INFRAALYZER 450 R + computerized filter spectrophotometer was connected to an IBM XT PC computer. All chemical and spectral data were stored in the computer's memory.
A PERKIN ELMER 139 UV/VIS spectrophotometer was used to determine the total alkaloids after distillation in an alkaline medium (CORESTA Standard Method No. 20). Digestion according to KJELDAHL on a TECATOR DS 20 system was used to determine the total nitrogen content. A total of 180 tobacco samples were selected from the material received at the chemical laboratory of the Tobacco institute Zagreb. The samples were selected with reference to the tobacco type (flue-cured) and with reference to the total nitrogen content (1.2-4.0%) and the total alkaloids (1.0-4.5%).

The samples were dried to a moisture content of 3-4% and ground on a WILLEY mill through an 0.2mm sieve. The optical parameter was log 10 (1/R) and was obtained by scanning through 19 filters according to the order of the firm TECHNICON called the "tobacco combination". All the samples were placed in a cuvette by the same operator to achieve the greatest possible uniformity. Each sample was scanned at least twice, and the analyses by classical methods were also repeated. The spectra (19 points) obtained by NIR and results of the classical chemical methods on 120 tobacco samples were stored in the computer data bank which were used to determine the calibration curves. The data for remaining 60 samples were used to check the calibration curves obtained. Step-wise multiple regression was used to select the appropriate wavelengths , and the ratio between the residual values and the residual standard deviation were used to eliminate inappropriate sample spectra.

RESULTS AND DISCUSSION

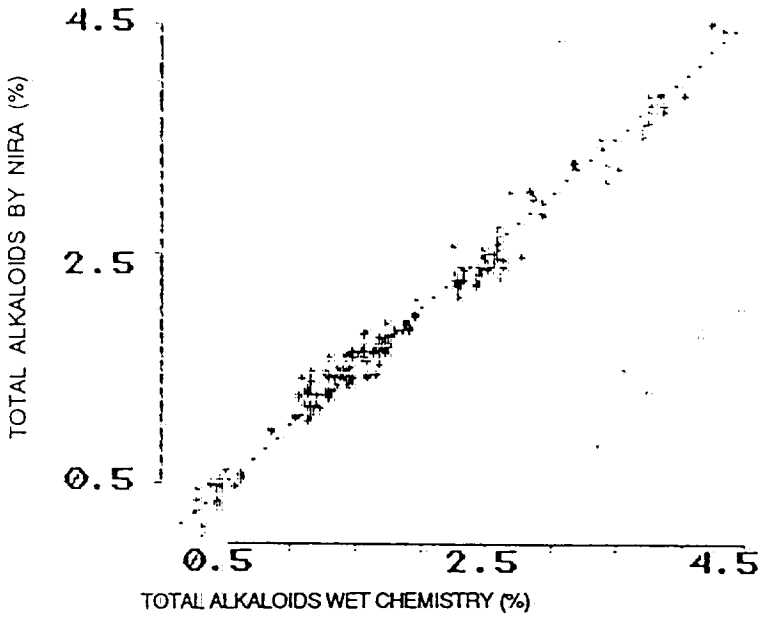
By plotting the spectral data and the wavelengths, spectra similar to the usual absorption spectra were obtained with peaks distributed in accordance with absorbing compound. Figure 1 shows the spectra of two samples with different chemical composition. In these two samples the difference in the content of total alkaloids was about 0.8 %, and in the content of total nitrogen about 0.5 %.

FIGURE 1
SPECTRAL DIFFERENCES FOR TWO SAMPLES OF FLUE CURED TOBACCO



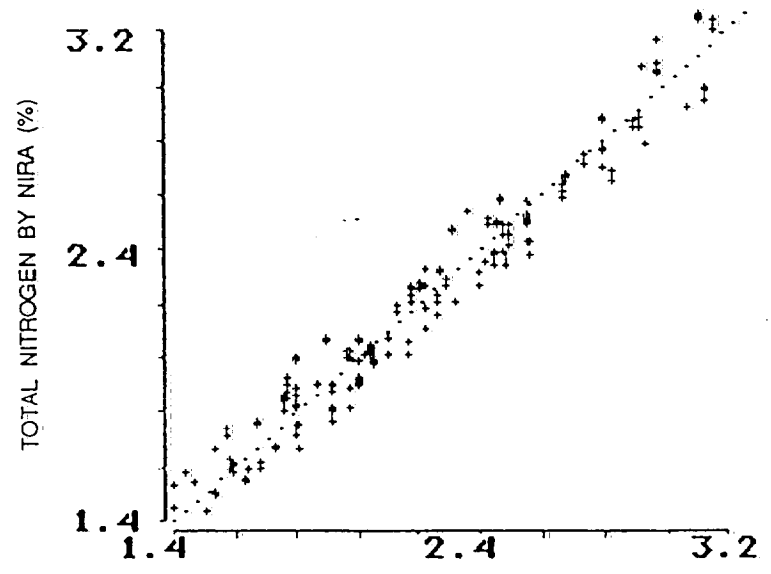
Figures 2 and 3 show the calibration curves obtained by the mathematical processing of data from the first data bank and the equations expressing the dependencies of the spectral changes and the concentrations of total alkaloids and total nitrogen in the tobacco samples.

FIGURE 2
CALIBRATION CURVE FOR TOTAL ALKALOIDS



TOTAL ALKALOIDS (%) = 1.862 + 1.77 (log 1/R)546 - 44.93 (log 1/R)2310 + 220.7 (log 1/R)2270 - 222.0 (log 1/R)2230 + 691.6 (log 1/R)1818 - 512.2 (log 1/R)1759 + 8.614 (log 1/R)1940 - 609.2 (log 1/R)1734 - 34.2 (log 1/R)1722 +807.8 (log 1/R)1680

FIGURE 3
CALIBRATION CURVE FOR TOTAL NITROGEN



TOTAL NITROGEN (%) = 4.941 + 1.677 (log 1/R)546 - 40.64 (log 1/R)2310 - 61.03 (log 1/R)2208 + 118.15 (log 1/R)2180 + 317.96 (log 1/R)1982 - 346.95 (log 1/R)1818 + 805.3 (log 1/R)1778 - 92.66 (log 1/R)1759 + + 13.8 (log 1/R)1940 + 560.32 (log 1/R)1734 - 617.1 (log 1/R)1722 - 34.15 (log 1/R)1445

The correlation coefficient on the total alkaloids curve was 0.9908 and the standard error of calibration was +/- 0.1681. The correlation coefficient for total nitrogen was 0.995 and the standard error of calibration was +/- 0.02.
A high degree of correlation was also achieved when the equations which were found were applied to the samples of the next set. The results are represented graphically in Figures 4 and 5.

FIGURE 4
RESULTS FROM TEST SET OF SAMPLES- TOTAL ALKALOIDS

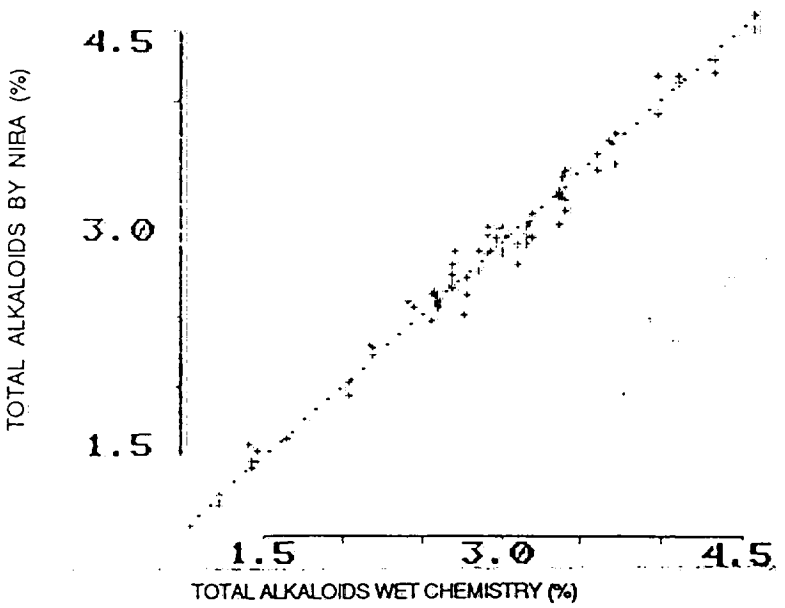
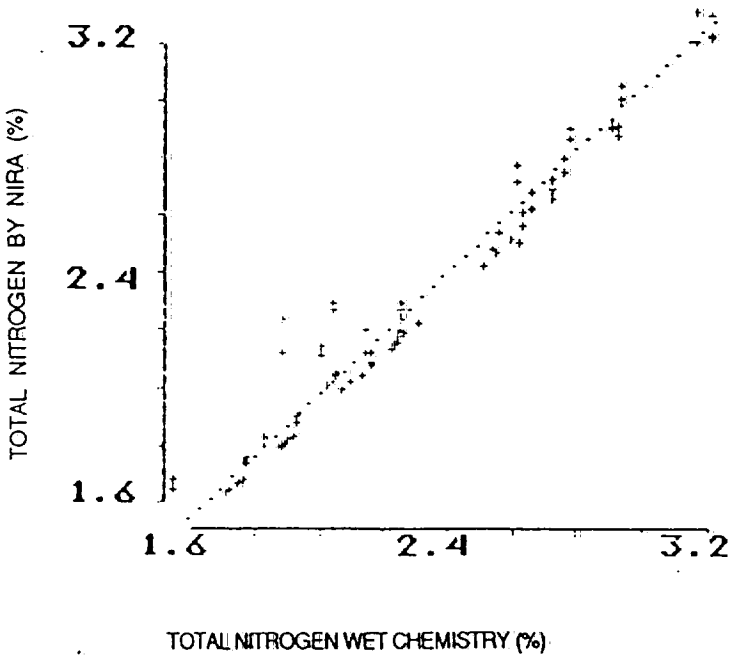


FIGURE 5
RESULTS FROM TEST SET OF SAMPLES-TOTAL NITROGEN



Five tobacco samples showed a significant discrepancy between the values obtained by wet chemistry and by spectrum analysis, amounting to 6.33% of the total number of samples tested (elimination criterion = RES/RSD ≥ 2).

In absolute values, the discrepancies amounted to up to 0.28%, and so the average error in determining the total alkaloids in the test set was +/- 6.2%. One part of the results is shown in Table 1.

The values for total nitrogen obtained in the same way are shown in Table 2, and a significant difference was observed in only 4 samples, which amounts to 5.1 % of the total number and so the scattering of the results ranged within a limit of +/- 5.5 %. Considering that in wet chemistry analysis the average error for total alkaloids ranged within a limit of 4.5 %, while the average error for total nitrogen ranged within a limit of 3 %, the results obtained by measurement of the amounts of these species by an INFRAALYZER are satisfactory, especially if the time and chemicals needed to perform analyses by wet chemistry are taken into account.

TABLE 1
CONTENT OF TOTAL ALKALOIDS IN THE TOBACCO SAMPLES
BY WET CHEMISTRY AND NIR ANALYSES

SAMPLE	TOTAL ALKALOIDS BY WET CHEMISTRY %	TOTAL ALKALOIDS BY NIR %	RESIDUAL VALUES WET CHEM. - NIR
1	3.54	3.61	-0.07
2	1.03	0.95	0.08
3	4.58	4.33	0.25
4	4.55	4.64	-0.09
5	0.61	0.57	0.04
6	0.45	0.40	0.05
7	1.74	1.66	0.08
8	2.59	2.51	0.08
9	3.27	3.31	-0.04
10	3.21	3.16	0.05
11	4.97	4.99	-0.02
12	5.03	4.88	0.15
13	5.52	5.55	-0.03
14	3.62	3.63	-0.01
15	3.51	3.53	-0.02
16	1.92	1.88	0.04
17	2.83	2.55	0.28
18	2.44	2.22	0.22

TABLE 2
CONTENT OF TOTAL NITROGEN IN THE TOBACCO SAMPLES
BY WET CHEMISTRY AND NIR ANALYSES

SAMPLE	TOTAL NITROGEN BY WET CHEMISTRY %	TOTAL NITROGEN BY NIR %	RESIDUAL VALUES WET CHEM. - NIR
1	1.76	1.82	-0.06
2	2.07	2.18	-0.11
3	1.67	1.78	-0.11
4	2.39	2.24	0.15
5	2.01	1.90	0.11
6	2.44	2.35	0.09
7	2.69	2.89	-0.20
8	2.40	2.68	-0.28
9	2.09	2.17	-0.08
10	1.72	1.80	-0.08
11	2.61	2.59	0.02
12	2.48	2.33	0.15
13	2.36	2.28	0.08
14	3.01	3.05	-0.04
15	2.32	2.39	-0.07
16	1.05	0.98	0.07
17	1.01	0.97	0.04
18	1.15	1.26	-0.11

It may be concluded that the content of total alkaloids and total nitrogen in tobacco samples can be determined with sufficient accuracy by the near infrared reflectance spectroscopy. The NIR technique is fast, reproducible, non-destructive and irreplaceable when a large number of samples has to be processed in a short time with low material and power consumption. Finally our experience has shown that for each year, i.e. each season and for each type of tobacco, the calibration has to be readjusted.

REFERENCES

1. W.F. McClure, F.G. Geisbrecht, W.W. Weeks. Using Fourier coefficients to estimate the chemical composition of tobacco. The 2nd annual NIRA Symposium, Technicon Corporation Tarrytown, New York, July 1982

2. W.F. McClure, K.H. Norris, W.W. Weeks. Rapid Spectrophotometric Analysis of the Chemical Composition of Tobacco, Part 1: Total Reducing Sugars. Beitr. Tabakforsch. 1977, 9, 13-18

3. A. Hamid, W.F. McClure, W.W. Weeks. Rapid Spectrophotometric Analysis of the Chemical Composition of Tobacco, Part 2: Total Alkaloids. Beitr. Tabakforsch. 1978, 9, 267-274

4. W.F. McClure, R.E. Williamson. Rapid Spectrophotometric Analysis of the Chemical Composition of Tobacco, Part 3: Polyphenoles. Beitr. Tabakforsch. 1982, 11,219-227